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Modeling Human Interindividual Variability in Metabolism and Risk: The Example of 4-Aminobiphenyl

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We investigate, through modeling, the impact of interindividual heterogeneity in the metabolism of 4-aminobiphenyl (ABP) and in physiological factors on human cancer risk: A physiological pharmacokinetic model was used to quantify the time course of the formation of the proximate carcinogen, N-hydroxy-4-ABP and the DNA-binding of the active species in the bladder. The metabolic and physiologic model parameters were randomly varied, via Monte Carlo simulations, to reproduce interindividual variability. The sampling means for most parameters were scaled from values developed by Kadlubar et al. (Cancer Res., **51**: 4371, 1991) for dogs; variances were obtained primarily from published human data (e.g., measurements of ABP N-oxidation, and arylamine N-acetylation in human liver tissue). In 500 simulations, theoretically representing 500 humans, DNA-adduct levels in the bladder of the most susceptible individuals are ten thousand times higher than for the least susceptible, and the 5th and 95th percentiles differ by a factor of 160. DNA binding for the most susceptible individual (with low urine pH, low N-acetylation and high N-oxidation activities) is theoretically one million-fold higher than for the least susceptible (with high urine pH, high N-acetylation and low N-oxidation activities). The simulations also suggest that the four factors contributing most significantly to interindividual differences in DNA-binding of ABP in human bladder are urine pH, ABP N-oxidation, ABP N-acetylation and urination frequency.

KEY WORDS: 4-Aminobiphenyl; toxicokinetics; interindividual variability; population heterogeneity; Monte Carlo Simulations.

INTRODUCTION

Human heterogeneity is not usually addressed in carcinogenesis modeling. Marked interindividual differences in the activation and detoxification of chemical carcinogens[1] suggest, however, that certain individuals may be at significantly greater risk of chemically induced cancer than the average individual. Increased susceptibility could be considerable when multiple metabolic pathways and critical interindividual differences in physiologic variables are involved. In this study, we modeled the bioactivation, detoxification, and other critical processes involved in bladder cancer induction by the arylamine 4-aminobiphenyl (ABP), including the individual variability in formation and activation of the proximate carcinogen, N-hydroxy-4-aminobiphenyl (N-OH-ABP), and in the fraction of ABP bound to DNA.

The proposed pathways for human metabolism of ABP are schematically described in Figure 1. Activation of ABP to N-OH-ABP is mediated by cytochrome P-450.[2–4] N-acetylation, catalyzed by arylamine N-acetyltransferase (NAT), acts as a detoxification pathway for ABP induced bladder cancer[5] and leads to the formation of N-acetyl-4-aminobiphenyl (N-Ac-ABP). Other detoxifying pathways may include cytochrome P-450 medi-

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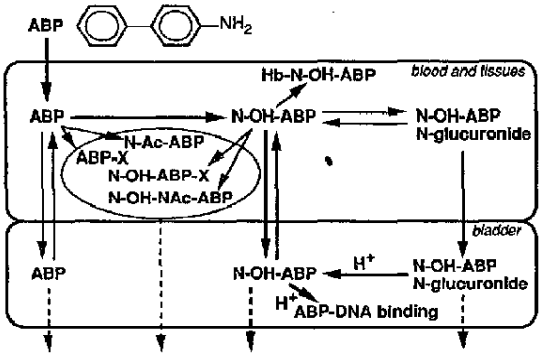

Fig. 1 Model of distribution and metabolism of ABP in humans. The major pathways for carcinogenesis are indicated by thicker lines. Dotted lines indicate excretion by intermittent voiding of the bladder. All processes represented by arrows are modeled by first order linear differential equations with rate parameters given in Table I.

ated ring hydroxylation,[6] N-glucuronidation[7,8] and N-sulfation.[9]

The proximate carcinogen, N-OH-ABP, is also metabolized in the liver, forming a N-OH-N-glucuronide conjugate[10] and possibly sulfate[11–13] and N-acetyl conjugates.[14] Some N-OH-ABP binds to hemoglobin (Hb) to form an Hb-N-OH-ABP adduct.[15,16] Unbound N-OH-ABP is believed to be activated in the bladder under mildly acidic conditions via the formation of an electrophilic intermediate which binds directly to urothelial DNA and presumably initiates the carcinogenic process.[4,17] Hydrolysis of the N-OH-N-glucuronide conjugate may also contribute to the level of N-OH-ABP in the bladder.

Many of the above activation and detoxification processes are mediated by enzymes exhibiting wide variation in activities. Heterogeneity in human cytochrome P-450IA2 activity, the isozyme involved in arylamine N-oxidation,[6] has been reported in both *in vitro* and *in vivo* studies.[6,18–20] Genetic polymorphism in N-acetylation is well established[21,22] and low acetylation has been associated with bladder cancer in a number of studies.[5,21,23] A study by Cartwright,[24] where occupational exposure to arylamine carcinogens has been documented, found nearly all exposed bladder cancer patients to be slow acetylators. Theoretically, interindividual variation in sulfation and/or glucuronidation[25–27] might also contribute to differential hepatic clearance and accumulation of N-OH-ABP in the bladder.

Finally, two physiological factors, urine pH and frequency of urination, appear to be risk factors for ABP-induced bladder cancer. Urine pH may determine the rate of formation of the reactive electrophile as well as the extent of hydrolysis of the N-glucuronide.[10] In dogs, frequency of urination has recently been suggested to be a critical factor in bladder exposure to N-OH-ABP.[28]

Because of the interdependence of these various processes, it is difficult to estimate the combined effect of their individual heterogeneity on human heterogeneity in ABP cancer susceptibility. To describe the possible extent of heterogeneity and to examine which factors may most contribute to it, we simulated the variability among humans by applying Monte Carlo simulations, a standard statistical technique,[29,30] to a pharmacokinetic model. Input parameters to the model were derived from the literature on human *in vitro* studies, or from dogs, in the absence of human data. ABP carcinogenesis is species-dependent and the dog appears to be the best model for humans.[31] The analysis is clearly constrained by the limited understanding and data on ABP pharmacokinetics in humans and on differences within populations.

METHODS

Pharmacokinetic Model

Our human model (Figure 1) is similar to that used by Kadlubar et al.[28] to mathematically describe ABP metabolism in dogs. All transport and most metabolic processes are described by first order kinetics. The body is divided into a central compartment and the bladder. ABP is absorbed through the gut wall. It then distributes to the central compartment (blood and tissues, in Figure 1) and can be excreted, unchanged, to the bladder compartment where efficient reabsorption may take place. ABP metabolism, the main elimination pathway, results in N-OH-ABP, N-Ac-ABP, or other metabolites, labeled ABP-X in the model. N-OH-ABP, in turn, binds to hemoglobin and other macromolecules, or may be transformed to N-OH-ABP-N-glucuronide, N-Ac-N-OH-ABP, or various metabolites (lumped together under the label N-OH-ABP-X). N-OH-ABP can also be transported to the bladder, where reabsorption can occur. A similar excretion/reabsorption pathway exists for N-OH-ABP-N-glucuronide which can also be hydrolyzed to N-OH-ABP, both in the central compartment and in the bladder. In urine this process is pH dependent.[28] Finally, N-OH-ABP can be activated in the bladder and bind to urothelial DNA, a pH-dependent process both in rate and extent.[10] The bladder empties periodically and its emptying is modeled as an instantaneous process.

Table I. Human Parameter Distributions Used in the Model

Parameter	Geometric mean[a]	CV(%)
Absorption		
K_ABP_abs[c]	0.033	50
K_ABP_urine→central[b]	0.033	20
K_N-OH_urine→central[b]	0.011	20
Distribution/metabolism		
K_ABP→Ac[c]	0.011	186
K_ABP→OH[d]	0.0083	180
K_ABP→X[e]	0.0054	50
K_N-OH-ABP→Ac[f]	0.0027	186
K_N-OH-ABP→X[g]	0.0083	50
K_N-OH-ABP→Hb[b]	0.0065	5
K_N-OH-ABP→Glu[b]	0.0036	50
K_hydrolysis_central[h]	0.0014	20
K_hydrolysis_urine[h]	795.0	5
K_activation[i]	670.0	5
fraction_bound[i]	0.0098	20
pH_urine[j]	6	10
Excretion		
K_ABP→urine[b]	0.00039	20
K_ABPX_exc[k]	0.011	20
K_N-OH-ABP→urine[k]	0.0018	20
K_N-OH_N-glu→urine[b]	0.00036	20
Urination Interval	270	*l*
Time of 1st urination	135	*m*

[a] Units: rates in min^{-1}, times in min.

[b] This parameter was scaled from dogs[28] to humans using the allometric equation $X_H = X_D \times (BW_H/BW_D)^{-0.3}$ [34], with $BW_D = 9$ kg and $BW_H = 70$ kg.

[c] The geometric mean was set at 2/3 of the "ABP→ABP-M" rate used for dogs[28], after scaling to human. At this value about 50% of ABP was acetylated. The CV was derived from human data[37] (see text).

[d] The geometric mean was set at half of the sum of the K_ABP→Ac and K_ABP→X rates, corresponding to 33% hydroxylation. The CV was derived from human data[6] (see text).

[e] The geometric mean was set at 1/3 the conversion rate of ABP to "other" metabolites for dogs[28], after scaling to human, corresponding to 17% conversion of ABP to ABP-X metabolites.

[f] The geometric mean was set at one fourth of the "N-OH-ABP→ABP-M" rate for dogs[28], after scaling to human. The CV was derived from human data[37] (see text).

[g] The geometric mean was set at three fourth of the rate of conversion of N-OH-ABP to "other" metabolites for dogs[28], after scaling to human.

[h] K_hydrolysis_urine (K) was obtained by estimating the rate constant of the hydrolysis (H^+ and OH^- mediated) of N-OH-ABP-N-glucuronide (i.e., fitting the equation % hydrolysis = $1 - \exp[-K \times 10^{-pH} + 10^{-p(OH)}) \times$ time]) to published data[10, chart 5]. A reaction including only H^+ does not describe the data adequately.

[i] K_activation (K) and fraction_bound (F) were obtained by fitting the equation %bound = $F \times (1 - pH \times \exp(-K \times 10^{-pH} \times$ time) to published data[10, chart 4]. This equation corresponds to the reactions H^+ + N-OH-ABP → N-OH^+-ABP and N-OH^+-ABP + DNA → DNA-N-OH^+-ABP, assuming that the second reaction is fast and that only a fraction F of the N-OH-ABP is available for reaction. This equation for %bound gave the best fit of several alternatives tried.

[j] Derived from fitting a lognormal distribution to human data from [10], with truncation interval [4.5, 7.5][53].

To account for the human heterogeneity in physiological and biochemical characteristics, the model parameter values were defined by statistical distributions rather than set values.[32,33] The specifications for these distributions are given in Table I and the derivation of these distributions is discussed in the next section.

Monte Carlo simulations,[30] were used to obtain the distribution of output variables (e.g., fraction of ABP bound to DNA in bladder). Each simulation was performed by first picking a random value for each model parameter, from its assigned distribution. The oral absorption of a bolus dose of 100 ng was simulated (our conclusions are not affected by the magnitude of the dose since linear kinetics are assumed). The time courses of output variables, including the area under the quantity-time curve (AUC) of N-OH-ABP and the quantity of ABP bound to DNA in the bladder, were recorded up to 72 hr. A total of 500 Monte Carlo simulations, theoretically representing 500 individuals, were performed. Additional simulations were performed to describe DNA binding in individuals with average and extreme physiologic and pharmacokinetic status (for Figure 3).

Characterization of Interindividual Variability in Parameters

All parameters, with the exception of the urination interval and the time of first urination after ABP exposure, were assumed to be lognormally distributed in the population (with mean μ and standard deviation SD_L). For clarity, Table I gives $\exp(\mu)$—the geometric mean—and the coefficient of variation (CV) in natural space. The SD_L used in the computations is related to the CV by the approximation $\exp(SD_L) = 1 + CV/100$. For most parameters, geometric means were obtained by taking the values for dogs given by Kadlubar et al.[28] and scaling them to humans. Volumes were assumed to scale with body weight and rates with body weight raised to the 0.7 power.[34] The rates reported for dogs sometimes apply to a group of metabolites; in such cases the rates were apportioned to maintain adequate mass-balance (see notes of Table I). CVs were derived primarily from human data, as explained below. For pathways and processes where no data on ABP or an arylamine congener

[k] The geometric mean is the sum of the urinary and fecal excretion rates of "ABP-M"[28], after scaling to human.

[l] Urination interval was sampled uniformly between the values 60 min and 480 min.

[m] Time of first urination was sampled uniformly between 0 and the sampled urination time.

were available, we used literature data describing the human variability in enzyme activities toward a range of xenobiotics to estimate CVs.

Direct Metabolism and Distribution of ABP

The variability in the rate of ABP N-oxidation (K_ABP→OH) was calculated from measurements in human liver microsomes from 22 organ donors[6] and was consistent with that of cytochrome P-450IA2 levels in hepatic tissue of healthy adults.[35] Values for ABP N-acetyltransferase activities in human liver cytosol have been reported in the literature for a small number of subjects.[36] However, distribution of acetyltransferase activities for 2-aminofluorene and the arylamine drug sulfamethazine[37] are also available and more extensive. The distributions of activities for these two arylamines are very similar in shape and spread.[38] We therefore used the data on 2-aminofluorene activities to obtain an estimate of the CV for ABP N-acetyltransferase activity (K_ABP→Ac).

The minor pathways (ring hydroxylation, N-glucuronidation and N-sulfation) are modeled as a group. Based on the data of Butler et al.,[6] ring hydroxylation quantitatively contributes very little to the metabolism of ABP. For ABP N-glucuronidation the findings of a 5-fold range in five individuals[8] most likely underestimates the variation due to small sample size. Other findings on the glucuronidation of various drugs indicate wider variation in activities.[25,27,39–42] Data on human N-sulfation of 2-naphthylamine in 20 individuals also suggests substantial variation, with a CV of 40% estimated from these data.[9] Estimates of the CV for N-sulfation of desipramine in humans were even higher.[43] We therefore adopted a CV of 50% for the rate of the combined minor pathways (K_ABP → X).

N-OH-ABP Metabolism

We have designated three pathways for N-OH-ABP metabolism: N-glucuronidation, N-acetylation, and X which combines all other pathways. Since direct data on N-glucuronidation of N-OH-ABP (rate K_N-OH-ABP → Glu) were limited,[10] we used literature data on glucuronidation of other xenobiotics by humans[8,27,39–42] to select a CV. There were relatively large variations for some xenobiotics (e.g., CV > 60%) while for others the range in activities was narrow (CV < 25%). Since the specific UDPGT isozyme(s) involved is unknown, we assumed moderate variability and selected a CV of 50%.

For the rate of N-OH-ABP N-acetylation (K_N-OH-ABP→Ac) we use the CV calculated for ABP N-acetylation. We assume that pathway X is comprised primarily of sulfate conjugation of N-OH-ABP.[11–13] Given the known genetic polymorphism for phenolsulfotransferase,[26] we selected a CV of 50% for K_N-OH-ABP → X.

Other Parameters

Data from 212 hospital patients[10] were used to calculate a CV of 10% for urine pH. The time interval between urinations was assumed to be uniformly distributed between 60 and 480 minutes. The average interval (270 minutes) is consistent with published data.[44] For the simulations the time of first urination after exposure to ABP was assumed to follow a uniform distribution over the time interval between zero and the sampled value of the urination frequency. For other pharmacokinetic and physiological parameters, a CV of 20% was assumed when variability appeared to be moderate and a CV of 5% when little or no variability was suggested. We assumed that Hb-N-OH-ABP binding was mostly a physical process and therefore, a CV of 5% was selected for the rate parameter K_N-OH–ABP → Hb. Similarly, we assumed that both activation of N-OH-ABP and hydrolysis of N-glucuronyl-N-OH-ABP in the bladder were purely physico-chemical processes and selected a CV of 5% for the corresponding parameters (K_activation and K_hydrolysis_urine).

RESULTS

Figures 2a and 2b are histograms of the AUC for N-OH-ABP in the bladder and of the fraction of ABP bound to DNA, respectively, after a simulated oral bolus dose of 100 ng. We ran 500 simulations to obtain these histograms, each simulation theoretically representing the exposure of an individual. The mean and 10th and 90th percentiles stabilized within that number of runs. We ran all simulations up to 72 hr and verified that after that time ABP was virtually eliminated from the body (in all cases less than 0.5% of the initial dose remained). Both distributions are asymmetrical and span 3 or 4 orders of magnitude. The 50% and 95% confidence intervals span approximately 1 and 2 orders of magnitude, respectively (the spread is larger for the fraction bound than for the AUC).

A further illustration of the potential differences in susceptibility among individuals is given in Figure 3,

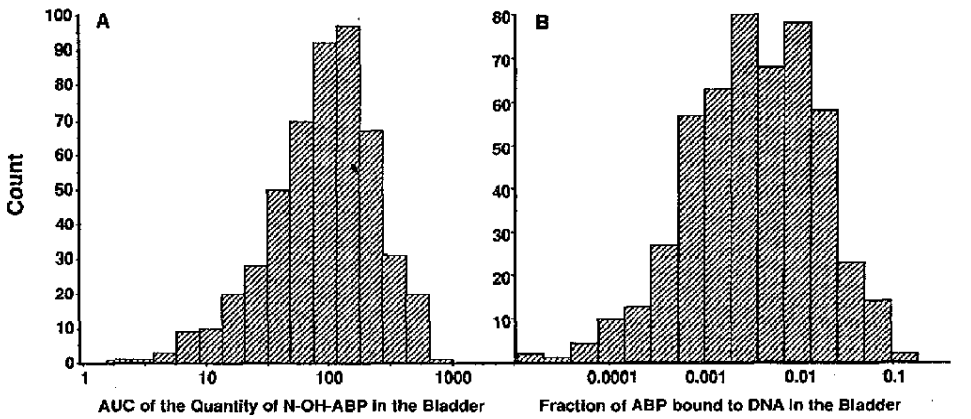

Fig. 2 Simulated distribution, for 500 subjects, of (A) the area under the quantity vs. time curve (AUC) for N-OH-ABP in the bladder (units: ng × min), and (B) the fraction of initial dose bound to DNA, in the bladder. In (A) the geometric mean is 89.5 ng × min, the arithmetic mean 135 ng × min, and the 95 pct confidence interval [7.6, 477]. In (B) the geometric mean is 0.0034, the arithmetic mean 0.0092, and the 95 pct confidence interval [1.3×10^{-4}, 0.053].

which presents the time course of the fraction of ABP bound to DNA in the bladder, for individuals with high and low N-acetylation or N-oxidation activities, and high or low urine pH. All other parameters were set to their geometric mean. There is a million-fold difference in DNA binding between the high pH, high N-acetylation, low N-oxidation condition and the other extreme, low pH, low N-acetylation, and high N-oxidation. The latter extreme differs from the median case by two orders of magnitude.

To rank the various parameters by order of importance, or their contribution to heterogeneity, the correlations between the Monte Carlo sampled parameter values and the model output of interest were examined.[45] The correlation coefficients depend on the intrinsic influence of each parameter on the outcome (which should differ for each outcome), and on the variability of the parameters in the population. The higher the correlation, the more dependent the outcome is on the parameter. Table II gives the correlation coefficients for log-transformed parameters and outputs, and the corresponding ranks for the influence of all model parameters on the AUC of N-OH-ABP and the fraction of ABP bound to DNA, both in the bladder. For the AUC of N-OH-ABP the most important parameters are the kinetic parameters K_ABP → OH and K_ABP → Ac, and the urination interval. Figure 4 shows the relationship between the AUC and these parameters. The smaller the degree of scatter in the points plotted, the greater the correlation and the impact of variability in the parameter on the AUC. For the fraction of ABP bound to DNA, the three most important parameters are

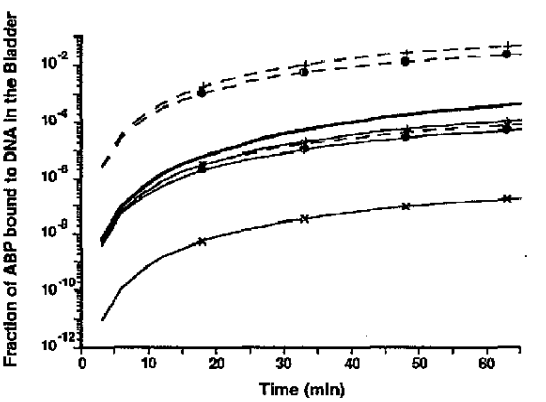

Fig. 3 Simulated time course of the fraction of initial dose bound to DNA, in the bladder, for various combinations of parameter values. Median conditions: —— (K_ABP → Ac = 0.011, K_ABP → OH = 0.0083, pH 6). Low acetylation, high oxidation, low pH: — + (K_ABP → Ac = 0.00047, K_ABP → OH = 0.18, pH 4.5). High acetylation, low oxidation, low pH: — -x- (K_ABP → Ac = 0.26, K_ABP → OH = 0.00038, pH 4.5). High acetylation, high oxidation, low pH: — -●- (K_ABP → Ac = 0.26, K_ABP → OH = 0.18, pH 4.5). Low acetylation, high oxidation, high pH: —+— (K_ABP → Ac = 0.00047, K_ABP → OH = 0.18, pH 7.5). High acetylation, low oxidation, high pH: -x- (K_ABP → Ac = 0.26, K_ABP → OH = 0.00038, pH 7.5). High acetylation, high oxidation, high pH: -●- (K_ABP → Ac = 0.26, K_ABP → OH = 0.18, pH 7.5).

urine pH, and again the kinetic parameters K_ABP → OH, and K_ABP → Ac (Figure 5); urination frequency comes fourth. The highest correlation is between fraction bound and urine pH, with a correlation coefficient of 0.754.

Table II. Sensitivity[a] of N-OH-ABP Bladder Exposure and DNA Damage to the Model Parameters

Parameter	AUC N-OH-ABP bladder		ABP bound to DNA	
	Correlation coefficient	Rank	Correlation coefficient	Rank
Absorption				
K_ABP_abs	−0.0776	9	−0.0847	12
K_ABP_urine→central	−0.0708	11	−0.1083	9
K_N-OH_urine→central	−0.0734	10	−0.0416	18
Distribution/metabolism				
K_ABP→Ac	−0.5325	2	−0.3808	3
K_ABP→OH	0.6538	1	0.4085	2
K_ABP→X	−0.1044	8	−0.0236	20
K_N-OHABP→Ac	−0.288	4	−0.1961	5
K_N-OHABP→X	−0.1498	7	−0.1293	8
K_N-OHABP→Hb	−0.051	14	−0.0258	19
K_N-OHABP→Glu	0.0545	13	0.0862	11
K_hydrolysis_central	0.0208	18	−0.0547	14
K_hydrolysis_urine	0.0634	12	0.0478	16
K_activation	−0.0036	20	0.0443	17
fraction_bound	0.0138	19	0.1386	6
pH_urine	−0.0358	16	0.7541	1
Excretion				
K_ABP→urine	0.0022	21	−0.0090	21
K_ABPX_exc	0.0302	17	0.0514	15
K_N-OHABP→urine	0.1704	6	0.0948	10
K_N-OH_N-glu→urine	0.0502	15	0.0735	13
Time of 1st urination	0.1795	5	0.1314	7

[a] The sign of the correlations is informative only for the highest correlation coefficients. For the low coefficients the finite sampling (500 runs) can lead to values whose signs are not significant (e.g., the coefficient for ABP absorption rate, which should correlate positively with the fraction of ABP bound to DNA, is negative).

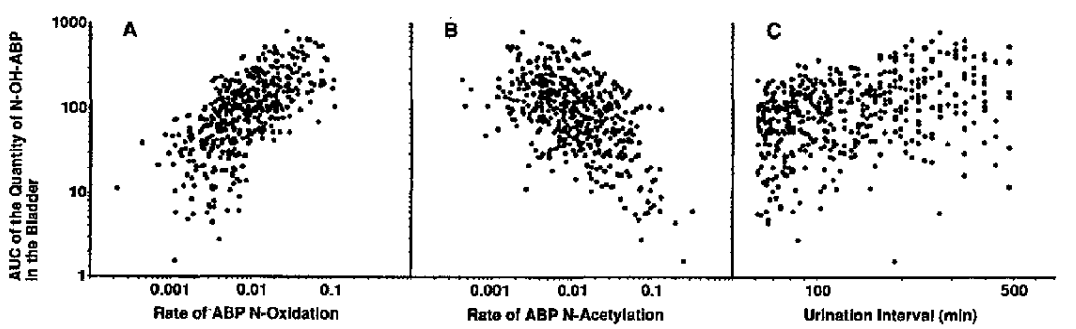

Fig. 4 Correlation between the area under the quantity vs. time curve for N-OH-ABP in the bladder and (A) the rate of ABP N-oxidation, (B) the rate of ABP N-acetylation, (C) the time interval between urinations, for 500 simulated subjects. The correlation coefficients are 0.654, −0.533, and 0.364, respectively.

DISCUSSION

These results show the importance of considering variability from multiple sources when assessing the susceptibility of individuals to ABP-induced cancer. These sources combine in a complex fashion and can be studied in detail by simulation. As can be seen from the results obtained, large differences can arise among individuals when a number of independent sources of variability exist. These results are consistent with studies of cigarette smokers where ABP-Hb adduct levels were lowest in individuals who were both fast acetylators and

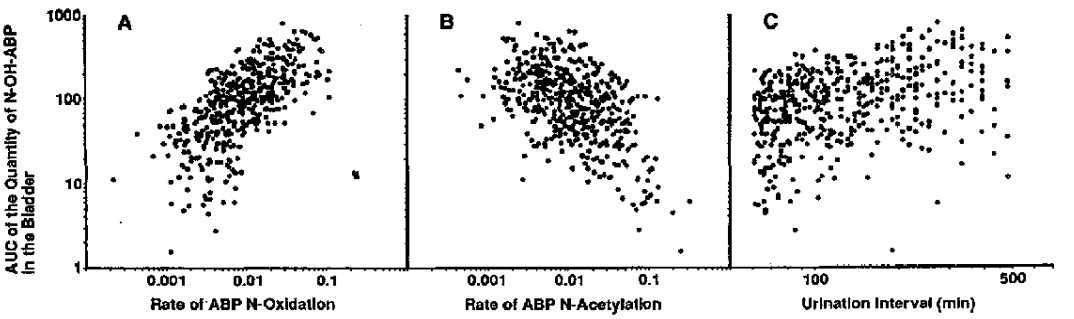

Fig. 5 Correlation between the fraction of initial dose bound to DNA in the bladder and (A) the rate of ABP N-oxidation, (B) the rate of ABP N-acetylation, (C) urine pH, for 500 simulated subjects. The correlation coefficients are 0.409, −0.381, 0.754, respectively.

slow/intermediate oxidizers.[46] For ABP, the formation of DNA-adducts in the bladder of the most susceptible individual is ten thousand times higher than for the least susceptible, in a sample of 500 simulations. Whether or not this translates into a four orders of magnitude difference in cancer risk is unknown. However, given the probable involvement of DNA lesions in the carcinogenicity of ABP, it is likely that large differences in DNA binding of ABP among individuals correspond to large interindividual differences in cancer susceptibility.

In addition to oxidation or acetylation activities, the physiological factors frequency of urination and urine pH were observed in these simulations to affect dramatically the extent of DNA binding. Frequency of urination appears to be very important in experimental studies in dogs as well.[16,28] Conversely, some processes were not observed to impact the binding of ABP to DNA. For example, the reactions involving N-OH-ABP N-glucuronide were found to be relatively unimportant. This could be explained by the fact that, according to our simulations, most of the compound is hydrolyzed in blood before having a chance to be eliminated in urine and therefore is not found in large quantities in the bladder.

Our predictions of interindividual differences, although large in value, may in fact understate the true human variability in ABP carcinogenesis since we investigated only a small number (even if significant) of the processes involved. Factors which could contribute to either over or underestimation of variabilities are model misspecification, unknown cross correlations of the parameters, or the mixing of measurement errors and intra-individual variability with inter-individual variability in the cross-sectional surveys from which the human data were obtained. In addition, data are available for characterizing variability on relatively few individuals.

The percentage of ABP metabolized by each pathway may be a subject of debate, and for many parameters where no data are available we took reasonable values. Minor pathways of metabolism may be much less important than we have assumed. Finally, the existence of many factors which have not been considered here, such as heterogeneity in DNA repair capacity,[47,48] or the presence of a low acetyltransferase activity in human urinary bladder cells,[49] may contribute further to the variability in the process believed to initiate ABP-induced bladder cancer. Had these factors been included, the estimated variability would had been higher.

The present data base to support this type of analysis for most chemicals, including ABP, is clearly limited. Human data are available for some critical metabolic pathways (e.g., oxidation) for ABP, but the numbers of subject studied are too small to give any confidence that the ranges reported are representative of the general population. The distributions of enzyme activities and of most physiological factors were assumed to be log-normal. This is a reasonable approximation for physiological variables, but the precision of the method depends primarily on the quality and amount of data available to define the empirical distributions of enzyme activities. The sample size of the populations investigated (22 subjects for cytochrome P-450 activities[6] and 35 subjects for N-acetyltransferase activities[37]) is insufficient to define with precision the shape and the tails or extremes of the distributions. The distributions below the 5th percentile and above the 95th percentile are poorly defined and statements regarding the most susceptible 1/10000 of the population would be inappropriate. The number of Monte Carlo simulations performed also influences the precision of the results, but in this case additional Monte Carlo simulations cannot make up for the lack of basic information on the distributions of enzyme

activities. Extending the simulations beyond 500 runs would not significantly improve the determination of the extremes or tails of the distributions because the available data are insufficient for defining the extremes of the underlying parameter distributions. Still, the results can provide insight on the extent by which risk may vary within a population.

We adapted to the case of humans a pharmacokinetic model proposed by Kadlubar et al.[28] to describe ABP pharmacokinetics in dogs. In the new model, acetylation of both ABP and N-OH-ABP, and pH-dependent binding of N-OH-ABP to DNA, are explicitly incorporated. The model is still a crude approximation of reality. For example, inter-species parameter scaling had to be used, and the assumption was made that only first order reactions were involved. A large literature indicates that cytochrome P-450 and acetyltransferase mediated transformations are complex, and may not be well modeled by first order reactions.[22,50–52] Nonetheless, for sufficiently low doses, linearity of the reactions is expected and the approximation should hold.

In conclusion, similarly exposed subjects or populations may have significantly different susceptibility to cancer due to differences in the metabolism/activation of carcinogens, and differences in physiological status. In typical carcinogenesis modeling it has been assumed that differences in susceptibility stem primarily from randomness of the occurrence of critical "hits" or DNA damage. Our results indicate the importance of considering inherent differences in human susceptibility to cancer as well. Note that most animal experiments, which use homogeneous populations provide little basis for assessing human variability. Upper confidence limits on risk, when derived from these experiments, are typically based on the assumption that each individual faces the same risk. Such estimates do not address inherent differences in human susceptibility.

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